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Dr. Ieva Ruks Politzer

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Xavier University of Louisiana
7325 Palmetto Street
New Orleans, LA 70125

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13. ABSTRACT *(Maximum 200 words)*

Cyclodextrin inclusion complexes were formed from p-nitroanilines and aniline analogs as well as from selected bimanes. Only in very few instances did complexation with cyclodextrins increase the second harmonic generation when compared to the parent compound (aniline/analog or bimane). Solid state UV reflectance spectroscopy was found to be a viable method for differentiating between solid cyclodextrin-guest inclusion complexes and cyclodextrin-guest physical admixtures. Isobestic points could be determined by solution UV absorption spectroscopy for a number of p-nitroaniline/analog aqueous solutions containing cyclodextrins. The TLC characteristics of p-nitroanilines and their analogs were greatly affected by the presence of cyclodextrins in the mobile phase. Stability constants were calculated from the TLC data.

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SECOND HARMONIC GENERATION FROM CYCLODEXTRIN INCLUSION COMPLEXES

FINAL REPORT

INCORPORATING THE REPORT FOR THE PERIOD
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DR. IEVA RUKS POLITZER

MARCH 30, 1995

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STATEMENT OF THE PROBLEM STUDIED

During the course of this project, we studied the effects of cyclodextrin inclusion complexation on various p-nitroanilines and their analogs as well as on selected bimanes. Solid inclusion complexes were prepared and examined for second harmonic generation by laser frequency doubling techniques. In the case of p-nitroanilines/analogs, inclusion complexes were prepared with α- and β-cyclodextrins. Only in very few instances did the complexation with cyclodextrins increase the second harmonic generation when compared to the parent compound. In the case of bimanes, inclusion complexes were prepared with β-cyclodextrin. In most cases, the second harmonic generation was only slightly increased by the complexation, as compared to the parent bimane itself. However, it was noted that for some of the bimanes, the second harmonic generation was initially considerably higher for the inclusion complex, but then decayed rapidly to the values reported.

Extensive studies were performed on the effects of β–, α–, γ- and hydroxypropyl-β-cyclodextrins on the thin layer chromatography of p-nitroanilines and their analogs using both silica gel and polyamide plates. In many cases, the presence of the cyclodextrins in the mobile phase resulted in dramatic changes on the TLC behavior of these compounds. Stability constants for the inclusion complexes were calculated from the TLC data.

The solid inclusion complexes were also examined by solid state UV reflectance spectroscopy. This spectroscopic method was found to be a viable means for differentiation between solid cyclodextrin-guest inclusion complexes and cyclodextrin-guest physical admixtures. Solution UV absorption spectroscopy was used to determine isobestic points for a number of p-nitroanilines and their analogs in aqueous solutions containing varying concentrations of cyclodextrins.

SUMMARY OF THE MOST IMPORTANT RESULTS

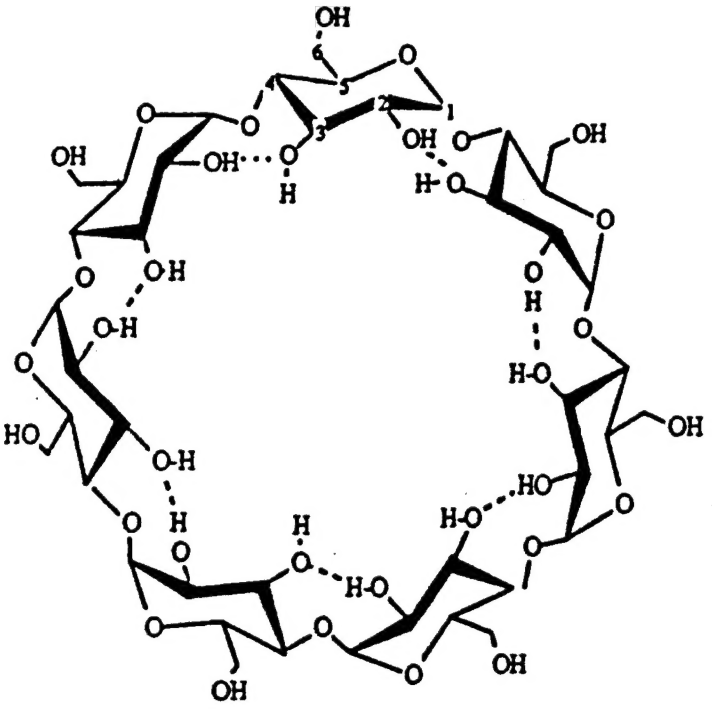
I. WORK WITH P-NITROANILINE AND ITS ANALOGS: COMPLEXES WITH CYCLODEXTRINS

1. Thin layer chromatography of p-nitroanilines and their analogs with cyclodextrins in the mobile phase

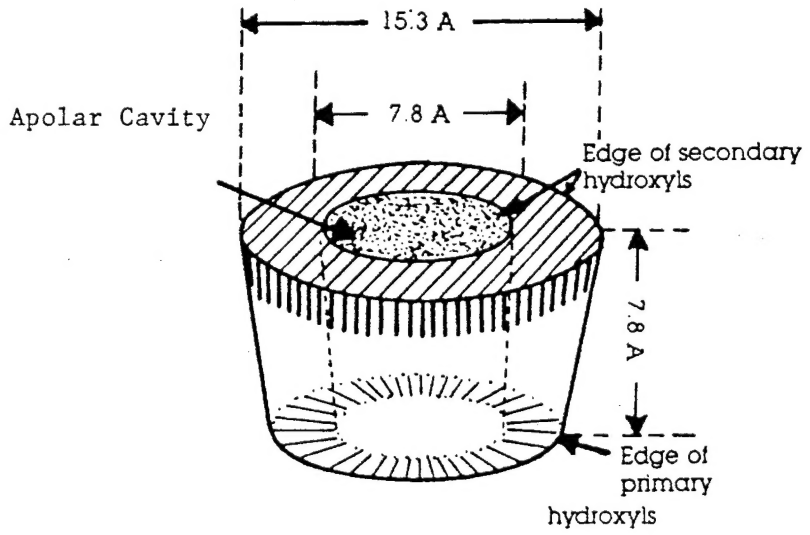
Cyclodextrins (CDs) are a homologous series of cycloamylases which are known for their ability to form inclusion complexes with a wide variety of guest molecules They are extensively used as stationary phase components in gas chromatography as well as stationary or mobile phase additives in liquid chromatography. The modest aqueous solubility of β-CD has somewhat limited its chromatographic applicability as a mobile phase component. However, the introduction of urea as a solubilizing agent for aqueous CD solutions, as well as the increased availability of substituted CDs, has opened the way for more extensive TLC applications. Figure 1 shows the chemical and dimensional structures for β-CD and the dimensions for the α- and γ-CDs.

Earlier work had indicated probable inclusion complexation between certain p-nitroanilines and CDs which resulted in induced second harmonic generation from the inclusion complexes. It seemed probable that the TLC characteristics of p-nitroanilines and their analogs would also be affected by CDs in the mobile phase. In this work, TLC studies were performed on p-nitroaniline (1), N-alkyl substituted-p-nitroanilines (2 and 3) and p-nitroaniline analogs with either the nitro-group or the amino-group replaced by other electron withdrawing or electron donating substituents respectively (4 - 9). Analogs also included 1-amino-4-nitronaphthalene (10), 4-amino-4'-nitrodiphenyl sulfide (11), 2-amino-6-nitrobenzothiazole (12) and 4-acetamidophenol (13). The structures for these p-nitroanilines and their analogs are shown in Figure 2. Both polyamide and silica gel were examined as solid support materials for the TLC plates. Aqueous mobile phases were used which contained alpha-, beta-, gamma- or hydroxypropyl-beta-CDs in the mobile phase. Urea was present as a solubilizer for the CDs, as needed. The effects of the CDs were studied on the TLC characteristics of the above mentioned p-nitroanilines and their analogs.

A comprehensive list of all of the p-nitroanilines and their analogs which were examined as guest compounds for the entire project is found in Table 1.



CHEMICAL STRUCTURE OF
β-CYCLODEXTRIN



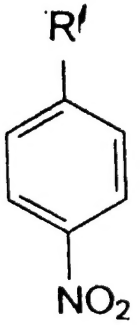
DIMENSIONAL STRUCTURE OF
β-CYCLODEXTRIN

MOLECULAR DIMENSIONS OF CYCLODEXTRINS

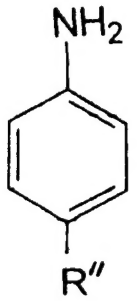
	α	β	γ
OUTER DIAMETER	13.7A^{O}	15.3A^{O}	16.9A^{O}
INNER DIAMETER	5.7A^{O}	7.8A^{O}	9.5A^{O}

FIGURE 1. STRUCTURES AND DIMENSIONS OF CYCLODEXTRINS.

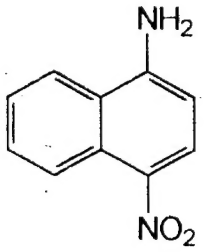
1 R = H
2 R = CH_3
3 R = CH_2CH_3



4 R' = OCH_3
6 R' = OH
8 R' = CH_3

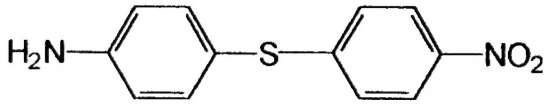


5 R'' = NC
7 R'' = COOH
9 R'' = COC_6H_5



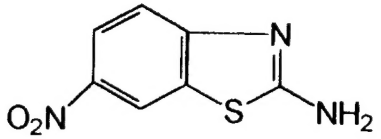
10

1-AMINO-4-NITRONAPHTHALENE



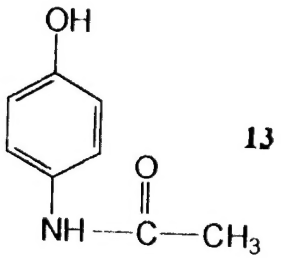
4-AMINO-4'-NITRODIPHENYL SULFIDE

11



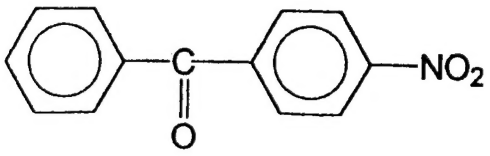
12

2-AMINO-6-NITROBENZO-THIAZOLE



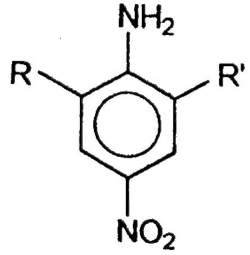
13

4-ACETAMIDOPHENOL



4- NITROBENZOPHENONE

14



15 R = H R' = CH_3
16 R = H R' = Cl
17 R = Cl R' = Cl

FIGURE 2. STRUCTURES OF SELECTED P-NITROANILINES AND THEIR ANALOGS.

TABLE 1. A LISTING OF SELECTED P-NITROANILINES AND THEIR ANALOGS USED IN THIS STUDY.

CPD #	COMPOUND NAME
1	PNA
2	N-MPNA
3	N-EPNA
4	4-NITROANISOLE
5	4-AMINOBENZONITRILE
6	4-NITROPHENOL
7	4-AMINOBENZOIC ACID
8	4-NITROTOLUENE
9	4-AMINOBENZOPHENONE
10	1-AMINO-4-NITRONAPTHALENE
11	4-AMINO-4'-NITRODIPHENYL SULFIDE
12	2-AMINO-6'-NITROBENZOTHIAZOLE
13	4-ACETAMIDOPHENOL
14	4-NITROBENZOPHENONE
15	2-METHYL-4-NITROANILINE
16	2-CHLORO-4-NITROANILINE
17	2,6-DICHLORO-4-NITROANILINE

Experimental

Alpha(α)-, beta(β)-, gamma(γ)-cyclodextrin (Advanced Separation Technologies, Whippany, NJ), hydroxypropyl-b-cyclodextrin, urea and all p-nitroanilines and their analogs (Aldrich Chemical Co., Milwaukee, WI), and solvents (Fisher Scientific Co., Raleigh, NC) were used as received without further purification. In-house demineralized water was used to prepare all aqueous solutions. The thin layer chromatography plates employed included Baker-flex polyamide 6-F (20 x 20 cm) and Baker-flex silica gel 1B-F (20 x 20 cm) plates. These plates were used as received. The mobile phase and stock solutions were prepared as described previously, . For spotting, a few microliters of the solute stock solutions were applied 2 cm from the lower edge of the plates. Ascending thin layer chromatography was performed in 27 x 8 x 25 cm rectangular chambers which were lined with mobile phase-soaked filter paper. The final positions of the solutes were located under UV-Vis light (Mineralight UVSL-58). Most of these compounds moved as distinct spots thus facilitating determination of retardation factors. The retardation factor (R_f) values were calculated using the formula:

R_f = distance compound travels/distance solvent travels.

Corresponding capacity factor (k') values were calculated using the relationship:

$$k' = (1 - R_f)/R_f.$$

The retardation factors were then related to the [CD] using the following equation (6):

$$R_f / (1- R_f) = (V_m /W_s) (1/ k'') [K_b [CD] +1]$$

where: R_f - the retardation factor

V_m - the volume of the mobile phase

W_s - the weight of the adsorbent in the bed

k" - the coefficient for the distribution of the solute between the bulk water of the mobile phase and the adsorbent of the stationary phase

K_b - the equilibrium binding (stability) constant for the solute-CD complex formed in the mobile phase (1:1 complexation)

Results and Discussion

Earlier work in our laboratories had shown that cyclodextrins (CDs) can be used to enhance the migration of various laser dyes on thin layer chromatography (TLC). In this study, we examined the effect of CDs on the TLC characteristics of selected p-nitroanilines and p-nitroaniline analogs. There did not appear to be any particular correlation between electron withdrawing or donating substituents on the p-nitroaniline analogs and their ability to influence chromatography or binding constants. Alpha-, beta-, gamma- and hydroxypropyl-beta-CDs were individually added (0.1 M CD concentration) to the aqueous urea mobile phases (8M urea with a-CD and 4M urea with all other CDs). Commercially available silica gel and polyamide plates served as solid supports.

The retardation factor (R_f) values were calculated and the results are shown in Table 2 for TLC on silica gel plates and in Table 3 for TLC on polyamide plates. With very few exceptions, mobile phases with CDs present (0.1M CD) resulted in enhanced migration for the compounds examined as compared to the migration of these compounds with urea only in the mobile phase. This suggests complexation between the p-nitroanilines and their analogs with most of the CDs examined.

Furthermore, the presence of urea in the mobile phase in combination with CDs (0.1M CD) was found to generally increase retardation factors for the p-nitroanilines and their analogs over those obtained with CDs only in the mobile phase. Figure 3 displays in bar-graph form the effects of urea on the R_f of p-nitroaniline (1) with various cyclodextrins (0.1M CD) in the mobile phase.

The retardation factors for the p-nitroanilines and their analogs were also found to vary with the concentration of the CD in the mobile phase. This is illustrated in Table 4 for compounds 1 - 3 using β-CD : 4 M urea in the mobile phase on silica gel as well as on polyamide plates. The β-CD concentration was varied over the range of 0 - 0.1 M. As can be seen, increased CD concentration in the mobile phase lead to increased compound

TABLE 2. EFFECTS OF UREA AND VARIOUS CYCLODEXTRINS ON THE Rf VALUES AND k' VALUES OF p-NITROANILINES AND THEIR ANALOGS ON SILICA GEL TLC PLATES

cpd #		O M CD 8M UREA	O M CD 4M UREA	alpha CD 0.1M CD NO UREA	alpha CD 0.1M CD 8M UREA	beta CD 0.1M CD NO UREA	beta CD 0.1M CD 4M UREA	gamma CD 0.1M CD NO UREA	gamma CD 0.1M CD 4M UREA	hydroxypropyl BCD 0.1M CD NO UREA	hydroxypropyl BCD 0.1M CD 4M UREA
1	Rf	0.56	0.47	0.81	0.91	0.71	0.86	0.62	0.73	0.70	0.82
	k'	0.79	1.13	0.23	0.10	0.41	0.16	0.61	0.37	0.43	0.22
2	Rf	0.38	0.26	0.86	0.92	0.57	0.88	0.32	0.58	0.72	0.77
	k'	1.63	2.85	0.16	0.09	0.75	0.14	2.12	0.72	0.39	0.30
3	Rf	0.18	0.15	0.77	0.80	0.42	0.64	0.20	0.23	0.62	0.07
	k'	4.56	5.67	0.30	0.25	1.38	0.56	4.00	3.35	0.61	13.29
4	Rf	0.00	0.08	0.68	0.39	0.00	0.00	0.00	0.00	0.61	0.68
	k'		11.50	0.47	1.56					0.64	0.47
5	Rf	0.71	0.69	0.84	0.90	0.75	0.89	0.79	0.83	0.79	0.89
	k'	0.41	0.45	0.19	0.11	0.33	0.12	0.27	0.20	0.27	0.12
6	Rf	0.33	0.58	0.75	0.62	0.69	0.85	0.68	0.77	0.01	0.91
	k'	2.03	0.72	0.33	0.62	0.45	0.18	0.45	0.30	99.00	0.10
7	Rf	0.88	0.84	0.94	0.96	0.87	0.96	0.90	0.91	0.81	0.92
	k'	0.14	0.19	0.06	0.04	0.15	0.04	0.11	0.10	0.23	0.09
8	Rf	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	k'										
9	Rf	0.24	0.13	0.00	0.28	0.82	0.78	0.64	0.63	0.72	0.86
	k'	3.17	6.69		2.57	0.22	0.28	0.56	0.59	0.39	0.16
10	Rf	0.20	0.08	0.00	0.20	0.07	0.29	0.00	0.28	0.64	0.71
	k'	4.00	11.50		4.00	13.29	2.45		2.57	0.56	0.41
11	Rf	0.00	0.00	0.06	0.05	0.50	0.77	0.05	0.06	0.70	0.77
	k'			15.67	19.00	1.00	0.30	19.00	15.67	0.43	0.30
12	Rf	0.53	0.24	0.90	0.84	0.68	0.92	0.48	0.56	no data available	
	k'	0.89	3.21	0.11	0.19	0.47	0.09	1.08	0.79		
13	Rf	0.94	0.85	0.82	0.88	0.81	0.87	0.15	0.90	0.81	0.86
	k'	0.06	0.18	0.22	0.14	0.23	0.15	5.67	0.11	0.23	0.16

TABLE 3. EFFECTS OF UREA AND VARIOUS CYCLODEXTRINS ON THE Rf VALUES AND k' VALUES OF P-NITROANILINES AND THEIR ANALOGS ON POLYAMIDE TLC PLATES

cpd #		O M CD 4M UREA	beta CD 0.1M CD NO UREA	beta CD 0.1M CD 4M UREA	hydroxypropyl BCD 0.1M CD NO UREA	hydroxypropyl BCD 0.1M CD 4M UREA
1	Rf	0.07	0.30	0.70	0.66	0.68
	k'	13.29	2.33	0.43	0.52	0.47
2	Rf	0.09	0.30	0.65	0.69	0.69
	k'	10.11	2.33	0.54	0.45	0.45
3	Rf	0.05	0.23	0.60	0.59	0.60
	k'	19.00	3.35	0.67	0.69	0.67
4	Rf	0.00	0.00	0.00	0.00	0.00
	k'					
5	Rf	0.24	0.38	0.49	0.62	0.64
	k'	3.17	1.63	1.04	0.61	0.56
6	Rf	0.09	0.18	0.34	0.40	0.49
	k'	10.11	4.56	1.94	1.50	1.04
7	Rf	0.28	0.51	0.66	0.79	0.73
	k'	2.57	0.96	0.52	0.27	0.37
8	Rf	0.00	0.00	0.00	0.00	0.00
	k'					
9	Rf	0.04	0.48	0.75	0.86	0.81
	k'	24.00	1.08	0.33	0.16	0.23
10	Rf	0.00	0.00	0.04	0.22	0.17
	k'			24.00	3.55	4.88
11	Rf	0.00	0.21	0.61	0.66	0.64
	k'		3.76	0.64	0.52	0.56
12	Rf	0.02	0.06	0.27	0.00	0.00
	k'	49.00	15.67	2.70		
13	Rf	0.21	0.54	0.57	0.67	0.62
	k'	3.76	0.85	0.75	0.49	0.61

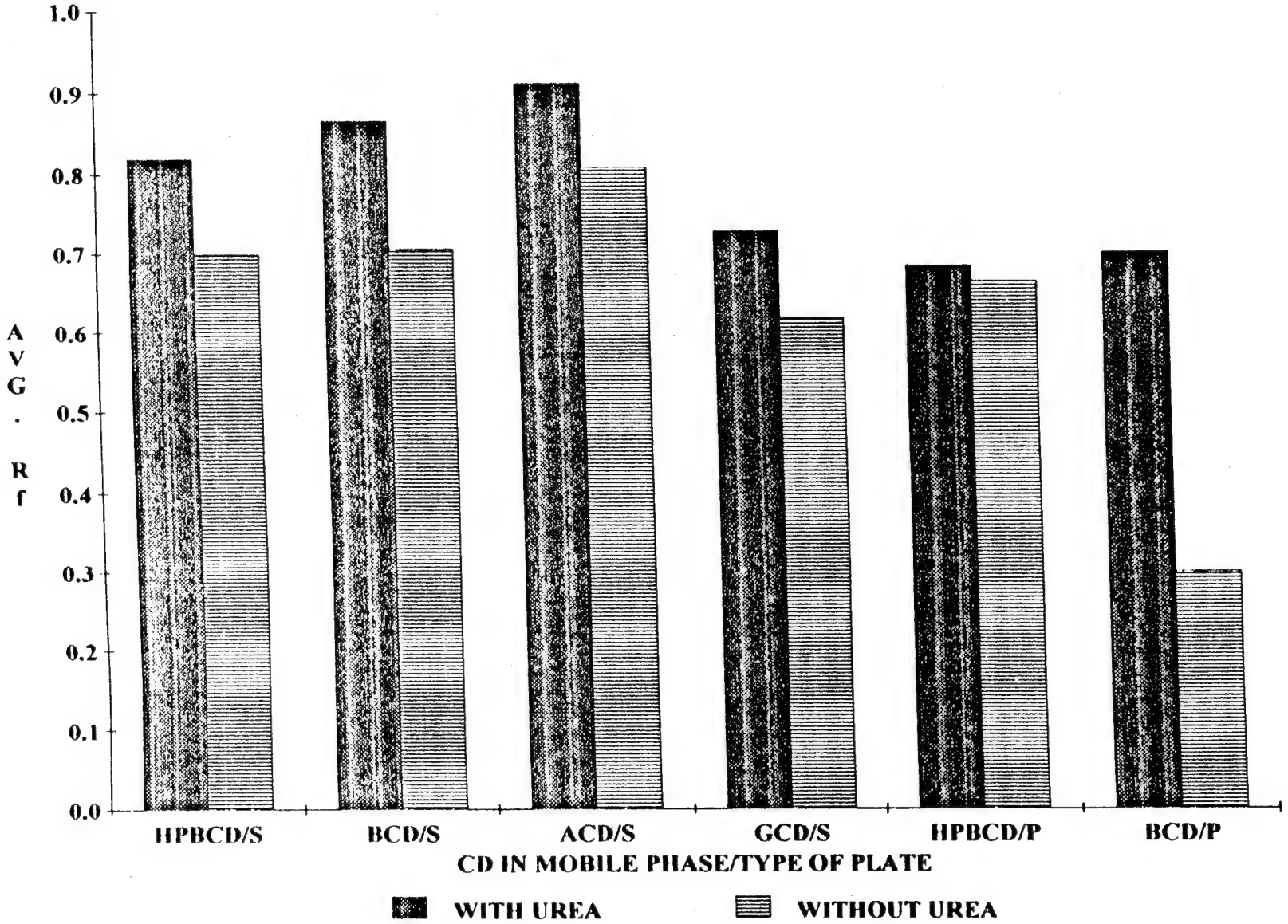


FIGURE 3. EFFECT OF UREA ON THE R_f OF P-NITROANILINE WITH VARIOUS CYCLODEXTRINS ([CD] = 0.1 M) IN THE MOBILE PHASE USING SILICA GEL (S) AND POLYAMIDE (P) PLATES .

migration on the silica gel as well as on the polyamide plates. Similar trends were observed for compounds 4 - 13 with all of the CDs used in this study.

TLC data can also be used to obtain a measure of the stability or binding constant for the inclusion complex formed between a compound and a particular CD in the mobile phase. For this purpose, retardation factors (R_fs) were obtained for each compound using a number of different CD concentrations in the mobile phase. In this study, the CD concentrations used were 0, 0.025, 0.05 and 0.1 M CD. Urea, 4 M, was present in the mobile phases when β-CD and hydroxypropyl-β-CD were used. The retardation factors were then related to the [CD] using the equation given in the Experimental section.

It was observed that plots of $R_f/(1-R_f)$ vs [CD] were fairly linear at low concentrations of CDs. These linear portions of the plots were used to obtain the values of slope/intercept or K_b, the equilibrium binding (stability) constants for the solute-CD complexes formed. Thus the K_b constants for compounds 1 - 13 were calculated for complexation with α–, β–, γ- and hydroxypropyl–β-CDs from R_f values obtained on silica gel plates. K_b constants for compounds 1 - 13 were also determined for complexation with β– and hydroxypropyl-β-CDs using R_f values obtained on polyamide plates. These results are presented in graphic form in Figure 4 .

The binding constants for complexes with α-CD were not included in this Figure since they were considerably out of line from the binding constants obtained for complexation with the other CDs used in this study. Thus, for example, the p-nitroanilines 1 - 3 showed outstandingly large binding constants for complexation with α-CD and a small to zero K_b was found for complexation of 4-aminobenzophenone (9) with α-CD (see Table 5). This was not altogether surprising, since α-CD has the smallest inner diameter of all of the CDs used in this study. Thus guest compound size limitations as well as different α-CD:guest compound complex stoichiometries may play a role in complexation involving α-CD.

TABLE 4. COMPARISON OF AVERAGE R_f VALUES OF P-NITROANILINES 1-3 ON SILICA GEL AND POLYAMIDE TLC PLATES WITH VARIOUS CONCENTRATIONS OF β-CYCLODEXTRIN IN AQUEOUS UREA MOBILE PHASES			
	SILICA PLATES		
4M UREA [βCD],M	PNA(1) AVG R_f	N-MPNA(2) AVG R_f	N-EPNA(3) AVG R_f
0	0.489	0.262	0.135
0.025	0.794	0.683	0.516
0.05	0.865	0.791	0.662
0.1	0.863	0.880	0.642
	POLYAMIDE PLATES		
4M UREA [βCD],M	PNA(1) AVG R_f	N-MPNA(2) AVG R_f	N-EPNA(3) AVG R_f
0	0.130	0.087	0.043
0.025	0.357	0.338	0.274
0.05	0.496	0.440	0.360
0.1	0.701	0.649	0.597

TABLE 5. STABILITY CONSTANTS (K_b, M^{-1}) FOR α-CYCLODEXTRIN-SOLUTE COMPLEXES IN THE MOBILE PHASE AS DETERMINED FROM TLC DATA ON SILICA GEL PLATES								
cpd #	1	2	3	4	5	6	7	8 - 13
K_b*, M^{-1}	3.4	11	14	0	1.2	.72	.53	0
*All K_b values should be multiplied by 10^2								

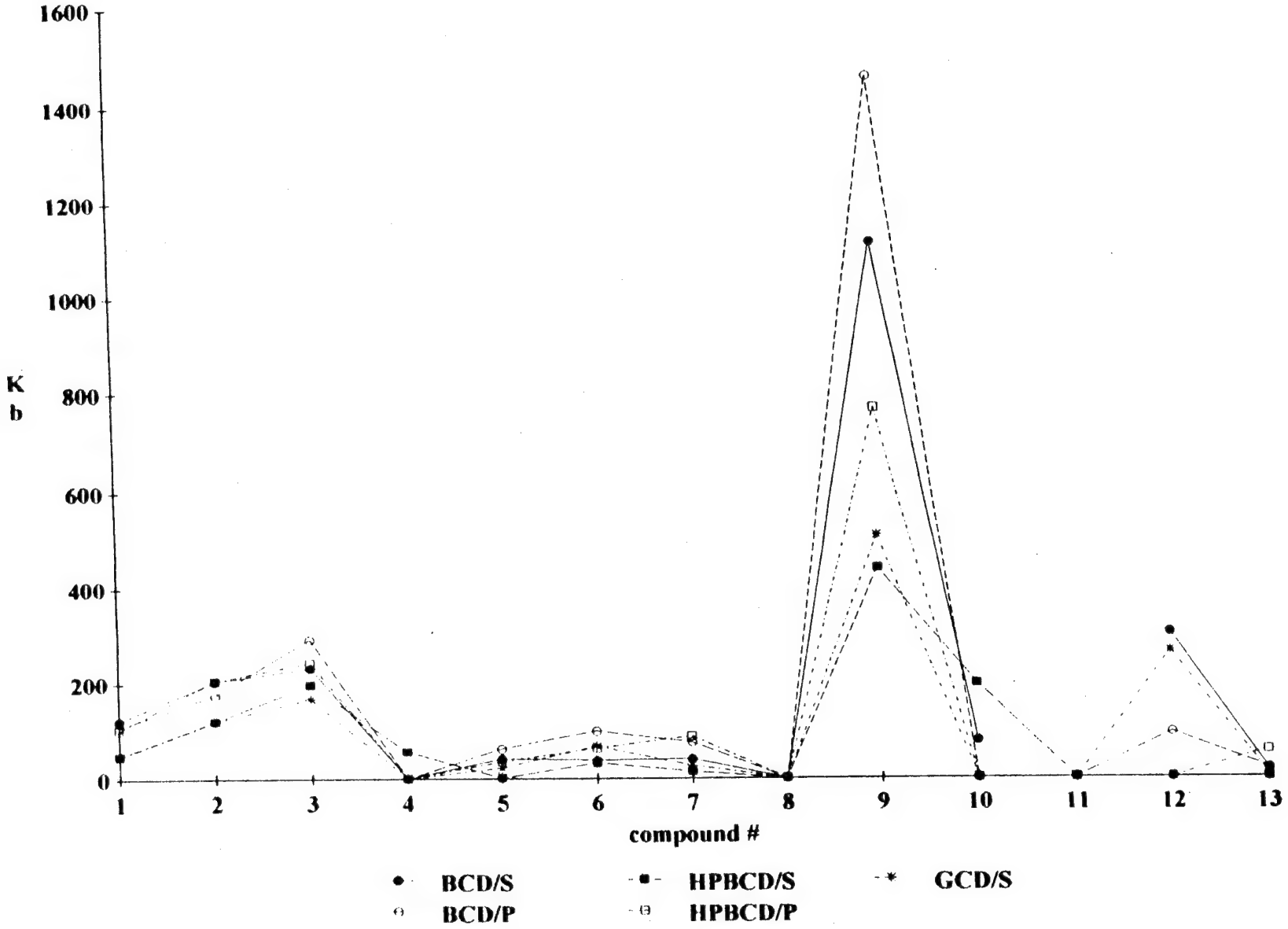


FIGURE 4. COMPARISON OF STABILITY CONSTANTS (Kb, M^{-1}) FOR CYCLODEXTRIN-SOLUTE COMPLEXES IN THE MOBILE PHASE AS DETERMINED FROM TLC DATA ON SILICA GEL (S) AND POLYAMIDE (P) PLATES. (HPBCD = hydroxypropyl-beta-cyclodextrin, BCD = beta-cyclodextrin, GCD = gamma-cyclodextrin)

Otherwise, as shown in Figure 4, remarkable overall similarity was noted for the trends in K_b values for the complexation of compounds 1 - 13 with β–, γ– and hydroxypropyl-β- CDs. Particularly noteworthy is the fact that similar trends for K_b values were observed on either polyamide or silica gel plates. In view of the ready availability and ease in handling of silica gel plates, this observation may be of practical consideration when use of TLC with CD-containing aqueous mobile phases is being considered.

As can be seen in Figure 4, it is evident that for most of the solutes examined (with the exception of compounds 9 and 12), the K_b values for binding of solutes to native β-CD are roughly the same as those observed for their binding to the derivatized hydroxypropyl-β-CD. This is important in view of the fact that many applications now are shifting useage from native β-CD to derivatized-β-CD. The finding that the binding interactions are similar enables one to approximate a binding constant for hydroxypropyl-β-CD if a binding constant is already available for native β-CD. In addition, this finding is in general agreement with luminescence-determined binding constants for native β-CD and the hydroxypropyl-β–CD derivatives for other series of solutes.

2. Analysis of cyclodextrin complexes with p-nitroanilines and their analogs using solid state UV reflectance spectroscopy.

Solid state cyclodextrin inclusion complexes have been distinguished from physical admixtures on the basis of X-ray powder diffraction patterns and DSC studies. We wish to report that solid state UV reflectance spectroscopy can also be used for this purpose. Solid state UV reflectance spectra were obtained for p-nitroaniline, its analogs, and their complexes with alpha- and beta-cyclodextrins as well as their physical mixtures with alpha- and beta-cyclodextrins (1:1 mole ratio).

Alpha- and beta-cyclodextrins showed nearly flat-line solid state UV reflectance spectra over the range 200 - 500 nm. First derivative spectra were also collected. Discrete and characteristic solid state UV spectra were obtained for each aniline analog, its cyclodextrin complex and its physical mixture with cyclodextrin. The spectra of complexes were generally different from the spectra of mixtures. In some cases, these differences could be seen even more markedly by taking first derivative spectra. Our results indicate that solid state UV reflectance spectroscopy is a viable method for differentiating between solid cyclodextrin inclusion complexes and physical mixtures.

Instrumentation used in this study included the following:

1) a Perkin Elmer Lambda 2 UV-VIS Spectrophotometer

2) Matching quartz cells (path= 0.5 mm)

3) RSA-PE-20 Reflectance Spectroscopy Accessory for the Perkin Elmer Lambda 2 Spectrophotometer

4) W.S.Tyler, Inc. sieve shaker-RX86

5) Fisher Scientific Co. -USA Std. Testing Sieve(ASTME-11specification); 60 mesh(Tyler equivalent)- particle size 250μm

6) Glenn Mills Inc.- Fritsch "pulverisette 2"-automatic laboratory mortar- grinder-type P2

Samples for this study were prepared in sets of four: 1)the parent compound,**2)** the solid inclusion complex of parent compound with cyclodextrin (α or β), **3)** the physical admixture of the parent compound with cyclodextrin (α or β), and **4)** the plain cyclodextrin.To take the actual spectra samples were then placed in matching quartz UV cells with a cell path of 0.5mm.(These were chosen because of the small amount of sample necessary to fill them). Reproducible spectra were obtained when larger cells were used.

1) Parent compounds were recrystallized from appropriate organic solvents,dried,mill ground, and sifted through the sieve(250 μm) using the sieve shaker.

2) Solid inclusion complexes (1:1 Mole ratios) were prepared by dissolving the appropriate amount guest (parent) compound($1.31x10^{-2}$moles of parent for α-cd complexes or $5.4x10^{-3}$moles of parent for β-cd complexes) in a minimum amount of diethyl ether (never less than 100ml) and layering this ether solution over a near saturated aqueous solution of the appropriate cyclodextrin[(concentrations = 12.7g α-cd/100ml H_2O($1.31x10^{-2}$moles) or 6.15g β-cd/300ml H_2O ($5.4x10^{-3}$moles)].* The two layer mixture was gently stirred overnight or longer until all of the ether had evaporated. The precipitate which formed slowly as the ether evaporated was collected by suction filtration and left on the vacuum overnight to dry. The dry complex was then ground by hand and sifted(250 μm) using the sieve shaker.

3)Physical admixtures (1:1 Mole ratios) were prepared by separate mill grinding of the parent compounds and of the cyclodextrins and sifting each substance separately (250μm) using the sieve shaker. The parent (guest) compound was then added to an equal molar amount of cyclodextrin and the two substances were hand mixed. This was done to decrease any complexation resulting from the intimate grinding of the mill.

4)Plain α- and β-cyclodextrin were ground and sifted as above.

* Note-

The actual molar amounts varied for α-cd and β-cd because of their water solubilities but the mole ratio between guest compound and cyclodextrin was always 1:1.

Solid state UV reflectance spectra were taken for compounds 1-17 and their complexes and physical admixtures with α–CD and ß-CD . Generally, discrete and characteristic spectra were obtained for each compound as well as for its complex and for its physical admixture with a particular CD. Since the solid state UV spectra peaks were very broad and shallow, it was difficult to assign lambda max values to them. However, the overall shapes and relative intensities of these spectra are highly reproducible. Thus, a particular guest, its cyclodextrin complex and its 1:1 guest:cyclodextrin physical admixture show the same relative patterns of scans each time, even when using different samples, different cells or different reflectance spheres.

For compounds 1- 8, 11 and 14 - 16, the complexes and admixtures with α- and/or ß-CD were blue shifted from the corresponding parent compound. For compounds 9,10 and 13; there was no blue shift and for compound 12, the complex was actually red shifted with both α - and β-CD. In most cases, the spectra of the complexes were different than the spectra of the admixtures. This was particularly evident for the complexes and admixtures formed with the guest compounds 1-4, 8, 9, 11, 12, 14-17 and α- or β-CDs. The complexes and admixtures were similar for guest compounds 5 and 6 with α–CD and for guest compound 7 with β-CD. In these cases it is speculated that instead of inclusion complexation, coprecipitation may have taken place.

In some cases, the differences between the UV scans for the parent (guest) compound, its complex and its admixture with cyclodextrin could be further accentuated by taking first derivative spectra. A striking example of this is shown for the complexes and admixtures formed with guest compound 12, (see Fig 5.).

In conclusion, our results indicate that in many cases, solid state UV reflectance spectroscopy is a viable method for differentiating between solid cyclodextrin-guest inclusion complexes and cyclodextrin-guest physical admixtures.

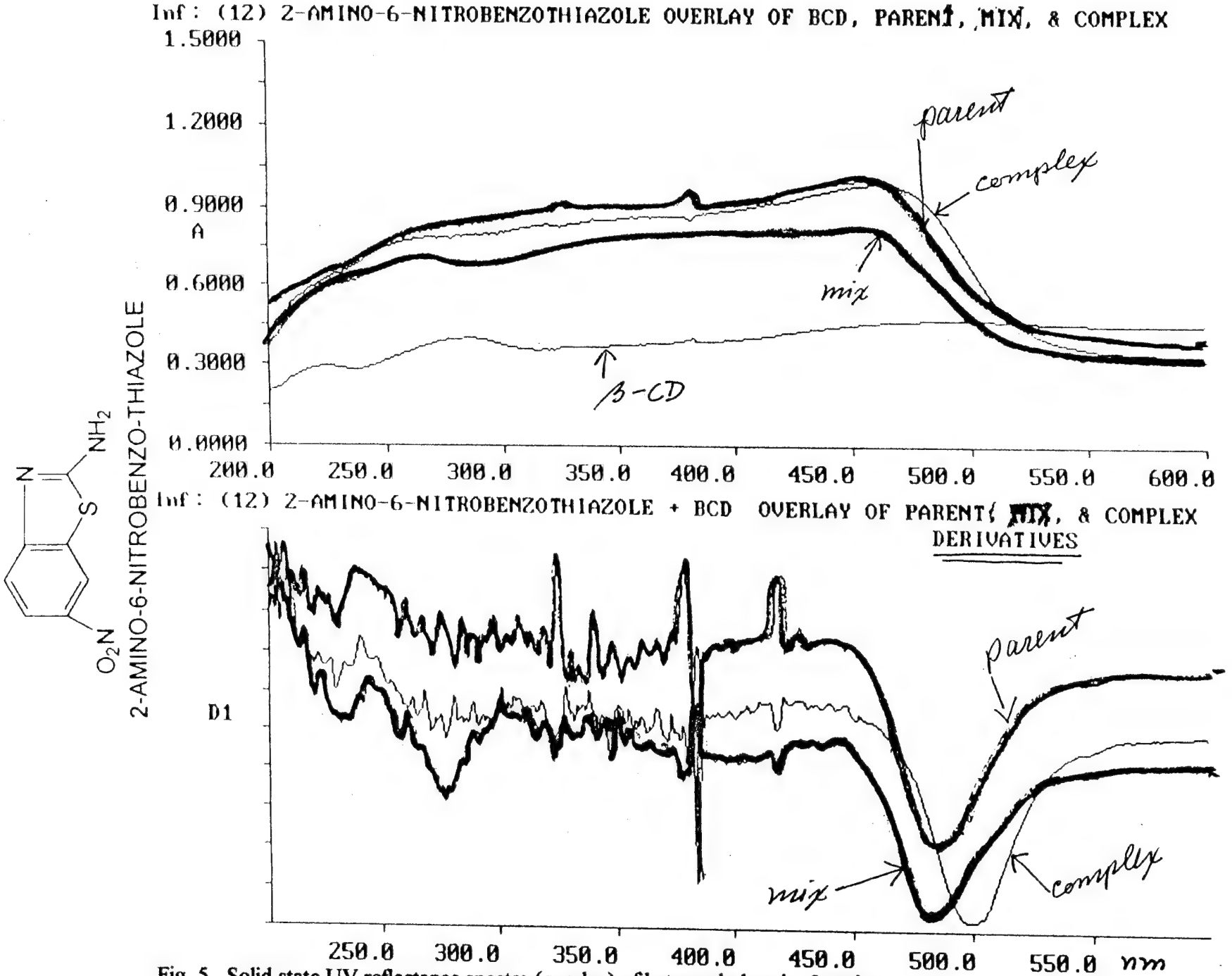


Fig. 5. Solid state UV reflectance spectra (overlay) of beta-cyclodextrin, 2-amino-6-nitrobenzothiazole, a mix of the two, a complex of the two and their first derivative spectra overlay.

3. Solution UV analyses and the determination of isobestic points.

Aqueous solutions of compounds 1 - 14 were examined by UV spectroscopy and their UV absobance was noted. These compounds were also examined for isobestic points over a beta-cyclodextrin concentration range of 0 - $1.4x10^{-2}$ M. The results are noted in Table 6. Compounds 13 and 14 were not sufficiently soluble to give good UV spectra. Isobestic points were obtained for compounds 1 - 3 and 7 - 10, 12 and possibly for compound 11. The other compounds did not show isobestic points. It was noted that compounds which showed the largest Kbs with beta -cyclodextrin (from TLC results) also showed isobestic points with beta-cyclodextrin solutions (from UV results). Compounds which had low Kb values generally did not show clear-cut isobestic points (compound 8 was an exception). Thus both Kb values and isobestic points indicate the existance of 1:1 complexes with beta-cyclodextrin for many of these compounds.

4. Second Harmonic Generation from p-nitroaniline analogs and their complexes and mixtures with cyclodextrins.

Solid inclusion complexes were prepared from p-nitroanilines and their analogs with α- and β-cyclodextrins. (A few complexes were also prepared using hydroxypropyl-β–cyclodextrin). For details on the preparation of the parent compound samples, the solid inclusion complexes and the physical admixtures, refer to page 19 of this report. The parent compounds, solid 1:1 inclusion complexes and physical 1:1 admixtures were then examined for second harmonic generation by laser frequency doubling techniques. All of the second harmonic generation measurements were performed at Howard University. The results are given relative to urea, used as a standard for comparison. As can be seen in Tables 7 and 8, only in very few instances did the complexation with cyclodextrins increase the second harmonic generation when compared to the parent compounds.

TABLE 6. UV ABSORPTION AND ISOBESTIC POINTS FOR ANILINE/ANALOG COMPOUNDS 1-17 IN AQUEOUS SOLUTIONS.

β-CYCLODEXTRIN CONCENTRATION RANGE 0 - 1.4 x 10^{-2}M

CPD #	COMPOUND NAME	λ max, nm	ISOBESTIC POINT OR OBSERVATION
1	4-NITROANLINE	380	371 nm
2	N-METHYL-4-NITROANILINE	480	397 nm
3	N-ETHYL-4-NITROANILINE	410	398 nm
4	4-NITROANISOLE	315	} no isobestic points found
5	4-AMINOBENZONITRILE	268	
6	4-NITROPHENOL	316	
7	4-AMINOBENZOIC ACID	278	278 nm
8	4-NITROTOLUENE	281	261 nm
9	4-AMINOBENZOPHENONE	331	323 nm
10	1-AMINO-4-NITRONAPTHALENE	441	461 nm
11	4-AMINO-4'-NITRODIPHENYL SULFIDE	351	385 nm - almost all absorbances cross here
12	2-AMINO-6'-NITROBENZOTHIAZOLE	358	370 nm
13	4-ACETAMIDOPHENOL		} water solubility too low to get good uv spectra
14	4-NITROBENZOPHENONE		
15	2-METHYL-4-NITROANILINE	385	
16	2-CHLORO-4-NITROANILINE	374	isobestic points not determined
17	2,6-DICHLORO-4-NITROANILINE	376	

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TABLE 7. SHG STUDIES ON ANILINE AND ITS ANALOGS WITH AND WITH β-CYCLODEXTRIN[a])

ANILINE or ANALOG (see Fig. 2)	SHG MEASUREMENTS RELATIVE TO UREA							
	Parent aniline or analog		1:1 Complex with β-cyclodextrin		1:1 Mixture with β-cyclodextrin		Residue left after complex formation	
	ratio	raw data	ratio	raw data	ratio	raw data	ratio	raw data
1	.01 (0)	-	20	-	5	-	-	-
2	0	-	.06	-	.01	-	-	-
3	.005	-	160	-	0	-	-	-
4	.009	4/450	.004	2/450	0	0/450	-	-
5	.03	15/450	.02	8/450	.01	6/450	.009	4/450
6	0	0/450	.01	5/450	.004	2/450	-	-
7	0	0/450	no complex formed		0	0/450	.09	40/450
8	0	0/450	.03	15/450	.01	3/450	-	-
9	40	400/10	1	450/450		650/70	-	100/450
10	.004	2/450	.004	2/450	.01	4/450	-	-
11	10 (8)	600/60 (400/50 recr)	.78	350/450	9.2	375/60	-	-
12	0	0/500	0	0/500	.01	4/450	-	-
13	0	-	0	-	0	-	0	-

SHG MEASUREMENTS RELATIVE TO UREA (CONTINUED)

ANILINE or ANALOG (see Fig. 2)	Parent aniline or analog		1:1 Complex with β-cyclodextrin		1:1 Mixture with β-cyclodextrin		Residue left after complex formation	
	ratio	raw data	ratio	raw data	ratio	raw data	ratio	raw data
14	0	0/100	0	0/100	0	0/100	0	0/100
15	7	700/100	.7	70/100	6	600/100	0	0/100
16	10	1000/100	0	0/100	3.5	350/100	0	0/100
17	0	0/100	0	0/100	0	0/100	0	0/100

a) All SHG studies performed at Howard University.

Note -native α-CD, β-CD and hydroxypropyl β-CD give an SHG 0/450 reltative to urea.

TABLE 8. SHG STUDIES ON ANILINE AND ITS ANALOGS WITH AND WITHOUT α-CYCLODEXTRIN[a])

SHG MEASUREMENTS RELATIVE TO UREA

ANILINE or ANALOG (see Fig. 2)	Parent aniline or analog		1:1 Complex with α-Cyclodextrin		1:1 Mixture with α-Cyclodextrin		Residue left after complex formation	
	ratio	raw data	ratio	raw data	ratio	raw data	ratio	raw data
1	.01	-	.005	-	0	-	-	-
2	0	-	0	-	0	-	-	-
3	.003	-	.005	-	0	-	-	-
4	0	0/450	0	0/500	0	0/500	-	-
5	0	15/450	.01	3/450	.02	8/450	-	-
6	0	0/450	0	-	0	-	-	-
7	0	0/450	.02	10/450	0	0/450	0	0/500
8	0	0/450	0	-	0	-	-	-
9	40	400/10	7.1	500/70	7.9	550/70	-	-
10	.004	2/450	0	-	0	-	-	-
11	4.67	1400/300	4.67	1400/300	3.33	1000/300	-	-
12	0	0/500	0	-	0	-	-	-
13	0	-	0	-	0	-	-	-

SHG STUDIES ON ANILINE AND ITS ANALOGS WITH AND WITHOUT α-CYCLODEXTRIN (CONTINUED)

ANILINE or ANALOG	Parent aniline or analog		1:1 Complex with α-Cyclodextrin		1:1 Mixture with α-Cyclodextrin		Residue left after complex formation	
	ratio	raw data	ratio	raw data	ratio	raw data	ratio	raw data
14	0	0/100 0/120	0	0/100 0/120	0	0/100 0/120	0	0/100 0/120
15	7	700/100	8	800/100	6	600/100	0	0/100
16	10	1000/100	.4	40/100	3	300/100	-	-
17	0	0/100	0	0/120	0	0/100	0	0/100

a) All SHG studies performed at Howard University.

II. WORK WITH CYCLODEXTRIN-BIMANE COMPLEXES

1. Fluorescence and UV studies of bimane complexes with cyclodextrins

Recently, efforts have been made to find ways to enhance the fluorescence of bimanes. Cyclodextrins, cycloamyloses which form inclusion complexes with a variety of molecules, have been shown to enhance the relative fluorescence emission and excitation intensity of bimanes. Under study were five bimanes: (1) Anti-(methyl, chloro) bimane, (2) Syn-(methyl, methyl) bimane, (3) Syn-(hydroxymethyl, methyl) bimane, (4) Syn-(methyl, acetoxyethyl) bimane and (5) Syn-(acetoxymethyl, methyl)bimane. When bimanes 3,4, and 5 were dissolved in water (10^{-5} M) and complexed with either α-, β-, or γ-cylcodextrin (10^{-2} M), it was found that γ-cyclodextrin enhanced fluorescence the most, while α-cyclodextrin actually reduced the intensity of the fluorescence emission. Addition of small volumes of t-butanol in all cases enhanced the fluorescence of aqueous solutions of the above bimanes. Addition of β-cyclodextrin in conjunction with t-butanol in most cases resulted in an even slightly greater enhancement of fluorescence.

SHORT DESCRIPTION OF BIMANES

Bimane is the common name given to a bicyclic heterocyclic ring system first systematically examined in 1980. Bimanes can be considered as bicyclic derivatives of pyrazole and they exist as *syn*- or *anti*- isomers. Most *syn*-bimanes exhibit striking and strong fluorescence in solution. Their quantum yields of fluorescence often range between 0.6 and 0.9. Generally, the *syn*-bimanes are weakly phosphorescent with quantum yields less than 0.009. The *anti*-bimanes are normally non-fluorescent. However, many show strong phosphorescence with quantum yields up to about 0.45.

The bimanes used in this study are shown in Fig. 6.

Instrumentation used in this study included the following:

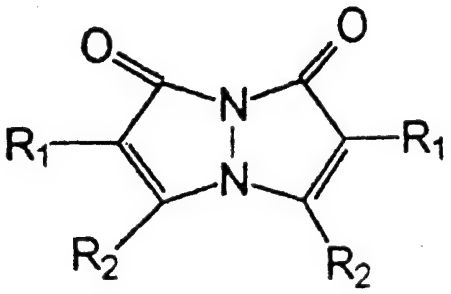
1) A Kontron SFM 25 spectrofluorometer equipped with a 150 W sealed Xenon lamp as the light source and a R 212 (200-650 nm range) photomultiplier sample detector.

2) A Perkin Elmer Lambda 2 UV-VIS Spectrophotometer

3) Matching quartz fluorometer cells (path = 10 mm)

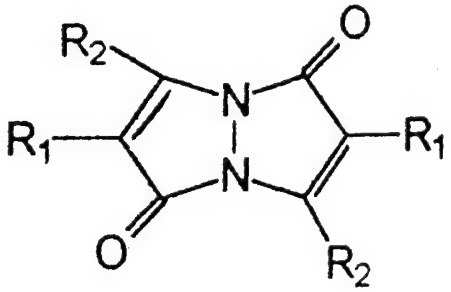
4) Matching quartz UV-VIS cells (path = 10 mm)

STRUCTURES OF BIMANES INVESTIGATED



syn - (R_2, R_1) bimane

R_1	R_2	Name
CH_3	CH_3	2 syn- (methyl, methyl) bimane
Cl	CH_3	6 syn- (methyl, Chloro) bimane
CH_3	CH_2OCOCH_3	5 syn- (acetoxymethyl, methyl) bimane
CH_3	CH_2OH	3 syn- (hydroxymethyl, methyl) bimane
$CH_2CH_2OCOCH_3$	CH_3	4 syn- (methyl, acetoxyethyl) bimane



anti - (R_2,R_1) bimane

R_1	R_2	Name
CH_3	CH_3	anti- (methyl, methyl) bimane
Cl	CH_3	anti- (methyl, Chloro) bimane

Fig. 6. Names and structures of selected bimanes.

A comparison study was performed on the effects of α-,β-,and γ-cyclodextrin on the UV absorption and fluorescence of aqueous solutions of bimanes using the three bimanes: *syn*-(methyl, acetoxyethyl) bimane, *syn*-(hydroxymethyl, methyl) bimane and *syn*-(acetoxymethyl, methyl) bimane. It was found that the addition of α-cyclodextrin to bimane solutions decreased or had no effect on the relative fluorescence intensity of the parent bimane solutions. β-Cyclodextrin addition generally resulted in a very slight increase in relative fluorescence intensity. Overall, the addition of γ-cyclodextrin enhanced the relative fluorescence intensity of the bimane solutions the most. **These results are summarized in TABLE 9 and are illustrated in Fig. 7 for the case of syn-(hydroxymethyl, methyl) bimane.**

The effects of the addition of α-, β- and γ-cyclodextrin on the UV absorption of bimane solutions were unexpected. γ-Cyclodextrin caused the largest decrease in the relative UV absorption of two of the three bimanes studied. β-Cyclodextrin caused similar, but less pronounced effects. Whereas α-cyclodextrin decreased the relative UV absorption of *syn*-(methyl, acetoxyethyl) bimane, and *syn*-(acetoxymethyl, methyl) bimane, it greatly increased the relative UV absorption of the *syn*-(hydroxymethyl, methyl) bimane. Ordinarily, fluorescence enhancement and UV enhancement go hand-in-hand. **These results are summarized in TABLE 10.**

Other research has indicated that addition of small amounts of alcohols, in conjunction with cyclodextrins, can greatly increase the fluorescence of certain organic compounds. Indeed, when a small amount of t-butanol was added to five aqueous bimane solutions, the relative fluorescence of the bimanes was markedly enhanced. Upon the addition of t-butanol and β-cyclodextrin together, the fluorescence intensity increased even more. UV absorption paralleled the fluorescence results for this aspect of the study. **The effects of t-butanol on fluorescence and UV absorption are indicated in TABLES 11 and 12 respectively.**

In summary, the results of this research indicate that γ-cyclodextrin is more effective than α- and β-cyclodextrins for enhancing the fluorescence of aqueous bimane solutions. Also, the addition of small amounts of t-butanol or t-butanol in conjunction with β-cyclodextrin, enhances the fluorescence intensity as well as UV absorption for aqueous bimane solutions.

Several of the bimanes were treated with varying concentrations of beta-cyclodextrin in attempts to find fluorescence isobestic points for the bimane-cyclodextrin complexes. Generally, the presence of beta-cyclodextrin induced some enhancement of bimane fluorescence. The changes in fluorescence were not sufficient to determine isobestic points, however. These results are summarized in Table 13.

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TABLE 9. COMPARISONS OF α, β AND γ CYCLODEXTRINS (10^{-2}M) ON THE FLUORESCENCE OF SELECTED BIMANES (10^{-5})*

CYCLODEXTRIN	syn-(CH_3, $CH_2CH_2OCOCH_3$)B λ_{em} = 457nm, λ_{ex} = 382nm		syn-(CH_2OCOCH_3, CH_3)B λ_{em} = 474m, λ_{ex} = 388nm		syn-(CH_2OH, CH_3)B λ_{em} = 474nm λ_{ex} = 388nm	
NONE	79.1%	78.1%	74.1%	75.3%	77.1%	76.2%
α-CD	78.7%	76.2%	74.1%	75.3%	74.6%	73.9%
β-CD	79.3%	78.2%	74.7%	75.6%	75.4%	73.8%
γ-CD	80.7%	79.1%	75.6%	76.3%	77.3%	75.4%

* Aqueous solutions were prepared using deionized water. Fluorescence determinations were made within 24 hrs after addition of cyclodextrin.

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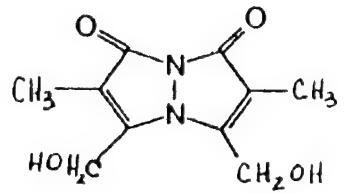
After comparing the fluorescence emission and excitation of the parent bimane solutions to the fluorescence emissions and excitations after the addition of α-, β-, or γ-cyclodextrin, the following trends were observed:

1. The addition of α-cylcodextrin to the parent bimane solutions either decreased the fluorescence emission and excitation or, as was the case with Syn-(CH_2OCOCH_3, CH_3) bimane, had no effect on fluorescence.

2. β-cyclodextrin addition caused a slight enhancement of the fluorescence emission and excitation of two of the three compounds studied. Fluorescence was decreased when β-cyclodextrin was added to the Syn-(CH_2OH, CH_3) bimane solution.

3. The relative fluorescence of the parent bimane solutions was most enhanced by the addition of γ-cyclodextrin. Syn-(CH_2OH, CH_3) bimane was the only compound whose relative fluorescence excitation decreased after the addition of γ-cyclodextrin.

Fig. 7. **Overlay--A Comparison of α-,β-, and γ- Cyclodextrin Addition on the Fluorescence Emissio**
Aqueous Syn-(CH_2OH, CH_3) Bimane



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SAMPLE:	Syn- (CH_2OH, CH_3) Bimane
CODE	Y.15.94
SCAN	EMISSION
LAMBDA SCALE	20 nm/cm
SCAN SPEED	100 nm/min
EXCITATION	388 nm
EMISSION	550 nm
CALIBRATION	75.0
HIGH VOLTAGE	314 V
BLANK	0.0
FACTOR	1.00
RESPONSE	8.0 sec

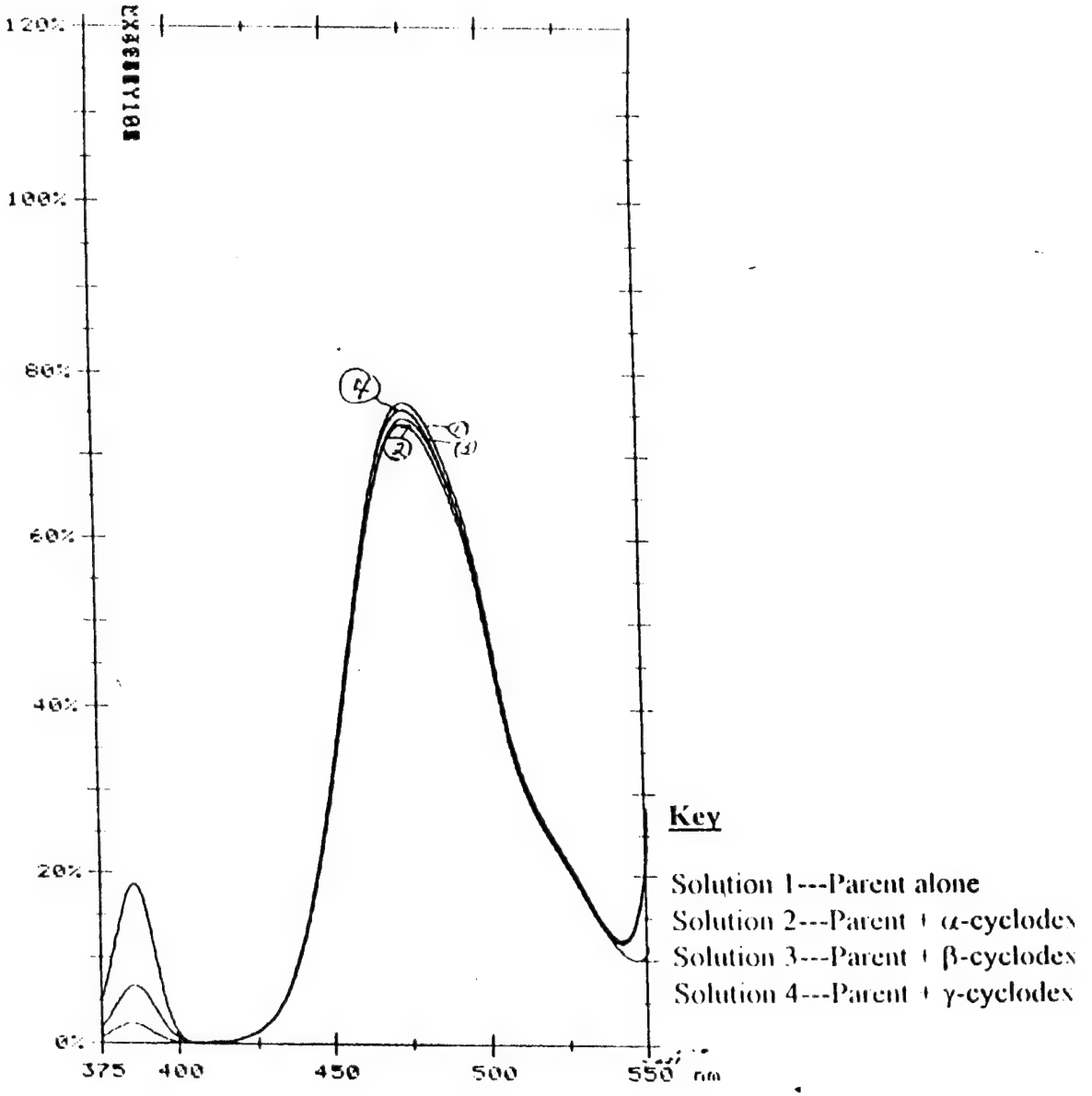

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TABLE 10. COMPARISONS OF α, β AND γ CYCLODEXTRINS (10^{-2}M) ON THE UV ABSORPTION OF SELECTED BIMANES (10^{-5})*

CYCLODEXTRIN	syn-(CH_3, $CH_2CH_2OCOCH_3$)B		syn-(CH_2OCOCH_3, CH_3)B		syn-(CH_2OH, CH_3)B	
	λ_{max}255	λ_{max}390	λ_{max}262	λ_{max}398	λ_{max}262	λ_{max}398
NONE	.0798	.0869	.0828	.0783	.0669	.0692
alpha-CD	.0617	.0753	.0802	.0749	.0948	.0835
beta-CD	.0529	.0750	.0823	.0760	.0980	.0861
gamma-CD	.0500	.0748	.0690	.0740	.0795	.0850

* Aqueous solutions were prepared using deionized water. UV determinations were made within 24 hrs after addition of cyclodextrins.

Comparisons of the UV absorption spectra of bimane solutions after the addition of α-, β-, or γ-cyclodextrin to the parent bimane solutions reveals that:

1. The addition of γ-cyclodextrin to solutions caused the greatest decrease in the relative UV absorption of Syn- (CH_3, $CH_2CH_2OCOCH_3$) bimane and Syn-(CH_2OCOCH_3, CH_3) bimane. However, the relative UV absorption of the Syn-(CH_2OH, CH_3) bimane solution was greatly enhanced. β-cyclodextrin addition had similar, but less drastic, effects on the relative UV absorption of the three bimane solutions.

2. While α-cyclodextrin addition decreased the relative UV absorption of the Syn-(CH_3, $CH_2CH_2OCOCH_3$) bimane and Syn-(CH_2OCOCH_3, CH_3) bimane solutions, it greatly increased the relative UV absorption of the Syn (CH_2OH, CH_3) bimane solution.

TABLE II.

Effect of the Addition of t-Butanol With and Without β-cyclodextrin (10^{-2}M) on the Fluorescence of Selected Aqueous Bimane Solutions

	PARENT BIMANE SOLUTIONS (10^{-5}M)									
	Syn-(CH_3,CH_3)B λ em 474, λex 383		Syn-(CH_2OCOCH_3,CH_3)B λ em 474, λ ex 388		Syn-($CH_2OH_1CH_3$)B λ em 474, λex 388		Syn(CH_3,$CH_2CH_2OCOCH_3$)B λem 455, λ ex 381		Anti(CH_3,Cl)B λ em 490, λex 321	
Parent + 10mL H_2O bimane	74.1%	74.7%	68.0%	69.0%	68.6%	67.7%	71.6%	71.1%	74.3%	75.5%
Parent bimane + H_2O + β-cyclodextrin	74.6	74.1	68.1	67.9	68.6	67.1	71.7	70.1	69.9	69.8
Parent bimane + 10mL t-Butanol	85.4	85.6	77.5	76.9	78.6	77.9	77.5	76.9	82.4	81.8
Parent bimane + t-Butanol + β-cyclodextrin	81.7	83.3	75.5	78.5	79.2	78.0	77.7	78.3	84.3	83.8

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The following trends were observed:

1. In all instances the addition of t-butanol and β-cyclodextrin enhanced the fluorescence excitation and emission of the bimane solutions more than the addition of water and β-cyclodextrin.

2. In all instances the addition of t-butanol alone enhanced fluorescence excitation and emission of the bimane solutions more than the addition of an equal volume of water.

3 In most cases, the addition of β-cyclodextrin in conjunction with t-butanol resulted in an even slightly greater relative enhancement of the fluorescence of the bimane solutions than the enhancement provided by t-butanol alone.

TABLE 12.

EFFECT OF ADDITION OF T-BUTANOL ON THE UV ABSORPTION OF AQUEOUS SOLUTIONS OF SELECTED BIMANES
a) WITH AND WITHOUT β-CYCLODEXTRIN

CONDITIONS	1B λ_{max} = 380nm	3B λ_{max} = 398nm	4B λ_{max} = 398nm	5B λ_{max} = 388nm	7B λ_{max} = 322nm
parent bimane 100ml, 10^{-2}M + 10ml H_2O	.0695	.0726	.0786	.0733	.2237
above b) + β-CD (10^{-2}M)	.0700	.0722	.0764	.0788	.2230
parent bimane 100ml, 10^{-5}M + 10ml t-butanol	.0712	.0682	.0798	.0856	.2418
above b) + β-CD (10^{-2}M)	0727	.0765	.0816	.0795	.2334

a) as above.

b) as above.

TABLE 13. **ATTEMPTS TO FIND FLUORESCENCE ISOSBESTIC POINTS FOR SELECTED BIMANES WITH β-CYCLODEXTRIN**

β-CD, M	syn-(CH_3,CH_3) bimane	anti- (CH_3, CH_3) bimane
aqueous solutions	10^{-5}M, λ_{ex} = 373nm, λ_{em} = 472-474nm no shifts in λ_{em} upon additon of β-cD % change in emission intensity	10^{-4}M, λ_{ex} = 334, λ_{em} no shifts in λ_{em} upon addition of β-CD % change in emission intensity
1.4×10^{-2}	enhancement 3%	enhancement 8%
1×10^{-2}	enhancement 3%	enhancement 4%
5×10^{-3}	enhancement 1%	enhancement 3%
1×10^{-3}	no change	enhancement 5%
5×10^{-4}	enhancement, less than 1%	quench 4%
2.5×10^{-4}	enhancement, less than 1%	quench 6%
1×10^{-4}	enhancement, less than 1%	quench 7%

β-CD, M	syn-(CH_2OCOCH_3) bimane	syn-(CH_3,$CH_2CH_2OCOCH_3$) bimane
aqueous solutions	10^{-4}M, λ_{ex} = 392nm, λ_{em} = 474nm no shifts in λ_{em} upon addition of β-CD % change in emission intensity	10^{-4}M, λ_{ex} = 382, λ_{em} = 456nm no shifts in λ_{em} upon addition of β-CD % change in emission intensity
1.4×10^{-2}	enhancement 10%	no change
1×10^{-2}	enhancement 4%	enhancement 5%
5×10^{-3}	enhancement 3%	enhancement 1%
1×10^{-3}	enhancement 10%	enhancement 3%
5×10^{-4}	enhancement 3%	enhancement 15%
2.5 x 10-4	enhancement 8%	enhancement 5%
1×10^{-4}	enhancement 5%	enhancement 2.5%

2. Second Harmonic Generation from bimanes and their complexes and mixtures with cyclodextrins.

Solid inclusion complexes were prepared from selected bimanes and β-cyclodextrin. Included were examples from both syn- and anti-bimane isomers. The sample preparation for the parent compounds, solid inclusion complexes and the physical admixtures paralleled closely the procedures described on page 19 of this report. The parent compounds, solid 1:1 inclusion complexes and physical 1:1 admixtures were then examined for second harmonic generation by laser frequency doubling techniques. All of the second harmonic generation measurements were determined at Howard University. The results are given relative to urea, which was used as the standard for comparison.

As can be seen in Table 14, in most cases, the second harmonic generation was only slightly increased by complexation with β-cyclodextrin, as compared to the second harmonic generation from the parent bimane itself. However, it was noted that for some of the bimanes, the second harmonic generation was initially considerably higher for the inclusion complex, but then decayed rapidly to the values reported.

TABLE 14. SHG STUDIES ON BIMANES WITH AND WITHOUT β-CYCLODEXTRIN[a)]

Bimane (B) Compound	parent B ratio	parent B raw data	1:1 B-β-cD complex ratio	1:1 B-β-cD complex raw data	1:1 B-β-cD mixture ratio	1:1 B-β-cD mixture raw data	residue from complex formation ratio	residue from complex formation raw data
syn-(CH_3, CH_3)B	1	400/400	.125	50 b)/400	.125	50b)/400	.35	140/400
syn-(CH_3Cl)B	.5	600/1200	.75	900/1200	.23	280/1200	.042	50/1200
syn-(CH_2OCOCH_3, CH_3)B	0	0/300	.067	20/300	0	0/300	0	0/300
syn-(CH_2OH, CH_3)B	0	0/300	0	0/300	0	0/300	0	0/300
syn-(CH_3, $CH_2CH_2OCOCH_3$)B	0	0/600	.33	200/600	0	0/600	0	0/600
anti-(CH_3CH_3)B	0	0/400	.15	60 b)/400	0	0/400	0	0/400
anti-(CH_3, Cl)B	0	0/300	.033	10/300	0	0/300	0	0/300

a) All SHG studies performed at Howard University. All SHG measurements are given relative to urea.

b) The SHG values for these materials start out much higher, but decrease to the reported values within a few seconds.

LIST OF ALL PUBLICATIONS AND TECHNICAL REPORTS

arising from research performed on ARO grant

DAAL 03-91-G-0226

I.R. Politzer, K.T. Crago, T. Hollin and M. Young, 1995.
TLC of p-nitroanilines and their analogs with cyclodextrins
in the mobile phase. J. Chromatographic Sci., Accepted for publication.

LIST OF ALL PARTICIPATING SCIENTIFIC PERSONNEL

Dr. Ieva R. Politzer
Mrs. Kathleen T. Crago
Mr, Keith Amos, B.S. Chemistry
Mr. Kyran Mitchell, B.S. Biology
Mr. Michael Young, B.S. Chemistry
Mr. Christopher Lemelle
Ms. Desiree Evans
Ms. Tiffani Hill
Ms. Shasa Dabner

BIBLIOGRAPHY - SAME AS LIST OF PUBLICATIONS